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NASAL SECRETIONS OF *NEISSERIA LACTAMICA* CARRIERS HAVE AN INHIBITORY EFFECT ON *NEISSERIA MENINGITIDIS* ATTACHMENT TO HUMAN OROEPITHELIAL CELLS

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Nasal secretions of volunteers colonized by N. lactamica *impaired the attachment of* N. lactamica *and of meningococci of groups A and B to oroepithelial cells. Bacterial adherence was found to be mediated by nonpiliated adhesins with antigen(s) which probably are shared by the strains tested. Although a strong attachment-inhibiting activity arises in their nasal secretions, volunteers remained colonized by* N. lactamica. *This evidence suggest that the eradication of* Neisseria *carriage is a multifactorial event.*

Key words: *Neisseria meningitidis* – *Neisseria lactamica* – carrier state – bacterial adhesiveness

Neisseria meningitidis may be isolated from the upper respiratory tract of a variable proportion of healthy humans. Meningococcal colonization results from a specific interaction between bacterial surface structures and receptors localized on the cellular membrane of the epithelial cells (Stephens & McGee, 1981; Stephens et al., 1982). *N. meningitidis* pili seems to play a significative role on the meningococcal adherence to oroepithelial cells "in vitro" (Stephens et al., 1982; Andrade, Wandersman & Santa Rosa, 1983) and to nasopharynx "in vivo" (Stephens & McGee, 1981). It was recently shown that the meningococci attached only to the nonciliated columnar cells of the nasopharynx and then penetrate into cell cytoplasm (Stephens, Hoffman & McGee, 1983). Therefore it is reasonable to suppose that the adhesive properties of meningococci also play an important role on the bacterial invasion from the upper respiratory mucosa through the bloodstream.

Neisseria lactamica shows close biochemical and antigenic similarities with the meningococci and some strains are serologically indistinguishable from serogroup B *N. meningitidis* (Hollis et al., 1970; Suassuna et al., 1975; Gold et al., 1978). It is also found in the human nasopharynx and occurs more frequently in children than in adults (Gold et al., 1978). *N. lactamica* has been rarely isolated from human disease and its occurence in pharyngitis, septicemia and meningitis seems to be associated with a debilited or immunocompromised host (Lauer & Fisher, 1976; Wilson & Overman, 1976; Cauduro, Mezzari & Dias, 1984). The antigenic similarity between *N. lactamica* and meningococci and the detection of bactericidal antibodies to meningococci in the serum of *N. lactamica* carriers suggest that nasopharyngeal carriage of *N. lactamica* may have a role in the development of natural immunity to meningococcal disease (Gold et al., 1978). On the other hand the low rates of meningococci recovery from the nasopharynx of *N. lactamica* carriers (Gold et al., 1978; Boia, Andrade & Camillo-Coura, manuscript in preparation) suggest that any kind of local immunity could interfere with meningococcal colonization of the nasopharyngeal mucosa. Inhibition of bacterial attachment to epithelial cells by secretory antibodies regulate the colonization of mucosal surfaces by other pathogenic microorganisms (Fubara & Freter, 1973).

The present report describes preliminary observations with volunteers harboring *N. lactamica* in their nasopharynx. The results indicate that the volunteers developed in their nasal secretions a strong attachment-inhibiting (AI) activity impairing *N. meningitidis* adherence to human oroepithelial cells.

MATERIALS AND METHODS

Five adult volunteers ranging in age from 22 to 36 years were selected. They were informed of possible risks and gave written consent to participate in the study. Nasopharyngeal cultures of all of them were negative for *N. meningitidis* and *N. lactamica* and their nasal secretions

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were found to be AI activity-negative for the bacterial strains tested. *N. lactamica* 244 (cross-reactive with meningococcus B antisera) was selected for intranasal inoculation of volunteers. *N. meningitidis* strains used in the adherence tests (21436 – serogroup A; 21536 – serogroup B) were isolated from CSF of patients with meningitis. *Neisseria* were identified and serogrouped (Corbett & Catlin, 1968; Lee et al., 1978; Morello, Janda & Bohnhoff, 1985) and overnight cultures in Columbia Agar Base (Difco) with 5% sheep blood (CSB) at 37°C in a humidified atmosphere of 5% CO_2 were suspended in GC Broth (Difco) with 20% glycerol and then stored at −25°C. Frozen specimens of each bacteria were thawed and used for each experiment. *N. lactamica* 244 was grown overnight in CSB (37°C, 5% CO_2) and a suspension with 10^9 colony-forming units (CFU)/ml was prepared in 0.85% saline. Each volunteer received 0.5 ml of bacterial suspension in each nostril twice, approximately 24 hr apart. The establishment and persistence of the carrier state was evaluated at the 2nd, 7th, 14th and 21st days post-inoculation (p.i.). Transoral nasopharyngeal swabs were collected (Hoeffler, 1974) and inoculated onto selective medium (Thayer & Martin, 1964).

Immediately after nasopharyngeal cultures nasal secretions and specimens of blood were also obtained. Sterile saline was instilled onto the nose until 6.0-8.0 ml of fluid was recovered and stored at −25°C. The nasal washes were thawed and 10x concentrated according to Wenzel et al. (1972).

Adherence tests were carried out with oroepithelial cells always collected from the same two donors, washed several times in Dulbecco phosphate-buffered saline (pH 7.2) (PBS-D) and adjusted with PBS-D to 10^5 cells/ml. Bacterial suspensions (10^7 CFU/ml) in PBS-D were prepared with bacteria grown for 18 hr on CSB (37°C 5% CO_2) and treated according to Stephens & McGee (1981) to avoid bacterial aggregates. Equal volumes (0.2 ml) of oroepithelial cell suspension and bacterial suspensions were incubated with 0.1 ml of nasal concentrate or with PBS-D in 24-well flat-bottom tissue culture plates (Costar) at 37°C for 15 min with circular agitation (120 r.p.m.). The mixture was then centrifuged five times at 200 xg for 2 min and the sediment of the last wash was extended on a glass slide, dried, fixed and stained with Gram's crystal violet. The total number of diplococci attached to 20 oroepithelial cells was determined in duplicate samples. Cells incubated in PBS-D without bacteria was used as a control of the indigenous bacteria attached to the cells.

For electron microscopy *Neisseria* colonies grown on CSB or on selective medium were gently dispersed into drops of Eagle MEM (Gibco). Parlodion-carbon-coated copper grids (200 mesh) were put floating over bacterial suspensions and successively transferred to 1% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4), distilled water, 1% PTA-10% saccharose-0.01% DMSO aqueous solution (pH 7.0) and again in distilled water. Grids were dried of excess moisture and examined with a Phillips EM-301 microscope.

For statistical analysis each bacterial strain had its' adherence indexes (from all volunteer's secretions collected at the same p.i. day) grouped and comparisons between groups were done by the Student's t test.

RESULTS AND DISCUSSION

Table I shows that *N. lactamica* carriage causes a detectable emergence of AI activity in the nasal secretions. The progressive increase in the antiadhesive effect would be compatible with the mounting of a local immune response (Fubara & Freter, 1973). AI activity was verified for both meningococcal strains tested although the strongest activity was directed against serogroup B strain, cross-reactive with *N. lactamica* 244 in slide agglutination tests. However, precocius rising in AI activity found for the serogroup B strain may be due to an anamnestic response associated to the current higher prevalence of B serogroup among meningococcal carriers in Rio de Janeiro (Boia, Andrade & Camillo-Coura, manuscript in preparation).

Although the AI activity induced in the nasal washings consistently impaired the attachment of meningococci and *N. lactamica* to oroepithelial cells, it did not result in the eradication of *N. lactamica* carriage. At the 21st p.i. day in spite of occurrence of an AI activity 3 to 10 times greater than the activity found in the preinoculation nasal secretions (Table I), all but one of the volunteers had *N. lactamica* in their nasopharynx (Table II). The only non-colonized volunteer (despite two unsuccessful inoculation attempts) already had a higher AI activity in his preinoculation nasal secretions in sharp contrast with the colonized volunteers (Table I). The eradication of the carrier state is probably dependent on other mechanisms besides a higher AI activity in the host nasal secretions.

TABLE I

Effect of nasal secretions of *N. lactamica* 244 carriers on *N. lactamica* and *N. meningitidis* attachment to oroepithelial cells.

Volunteers	Microorganisms*	Adherence to oroepithelial cells			
		nasal secretions from post-inoculation days:			
		0	7th	14th	21st
J.A.	a	4.8**	ND	3.3	0.5
	b	9.0	ND	0.3	0.3
	c	7.1	ND	1.2	0.5
C.C.	a	1.8	1.5	2.3	0.6
	b	7.6	1.3	0.4	0.2
	c	1.6	2.0	0.9	0.7
M.B.	a	5.5	1.4	1.7	0.6
	b	3.4	1.1	0.5	0.2
	c	4.8	1.2	1.0	0.6
M.C.	a	5.1	1.9	4.0	0.5
	b	4.4	1.1	0.3	0.5
	c	6.1	1.1	1.1	0.5
E.N.	a	0.2	ND	0.15	0.7
	b	0.8	ND	0.9	0.1
	c	0.6	ND	2.0	0.5

* a: *N. lactamica* 244; b: *N. meningitidis* 21536 (group B); c: *N. meningitidis* 21436 (group A).

**adherence index (see text); ND = not determined

Statistical significances: *N. lactamica* 244 : 0–21st p.i. day = $P < 0.01$; *N. meningitidis* 21536 : 0–7th/–14th/–21st p.i. days = $P < 0.001$; *N. meningitidis* 21436 : 0–7th p.i. day = not significant; 0–14th p.i. day = $P < 0.1$; 0–21st p.i. day = $P < 0.01$

TABLE II

Establishment and persistence of *N. lactamica* 244 carriage in volunteers

Volunteers	Recovery of *N. lactamica* 244				
	Post - inoculation days:				
	0	2nd	7th	14th	21st
J.A.	–	+	+	+	+
C.C.	–	+	+	+	+
M.B.	–	+	+	+	+
M.C.	–	+	+	+	+
E.N.	–	–	–	–	–

N. meningitidis pili play a role in the nasopharyngeal colonization and in the bacterial attachment to oroepithelial cells (Stephens & McGee, 1981; Stephens et al., 1982, Andrade, Wandersman & Santa Rosa, 1983; Stephens, Hoffman & McGee, 1983). However, both *N. lactamica* 244 (Fig. 1a, 1b) and meningococcal strains 21436 and 21536 were completely devoid of pili. Therefore, nonpiliated adhesins may assist in the bacterial attachment to oroepithelial cells and perhaps in the nasopharyngeal colonization by *N. lactamica* and *N. meningitidis*. Some degree of antigenic similarity between these adhesins could be inferred from the cross-reacting AI activity shown by the adherence tests.

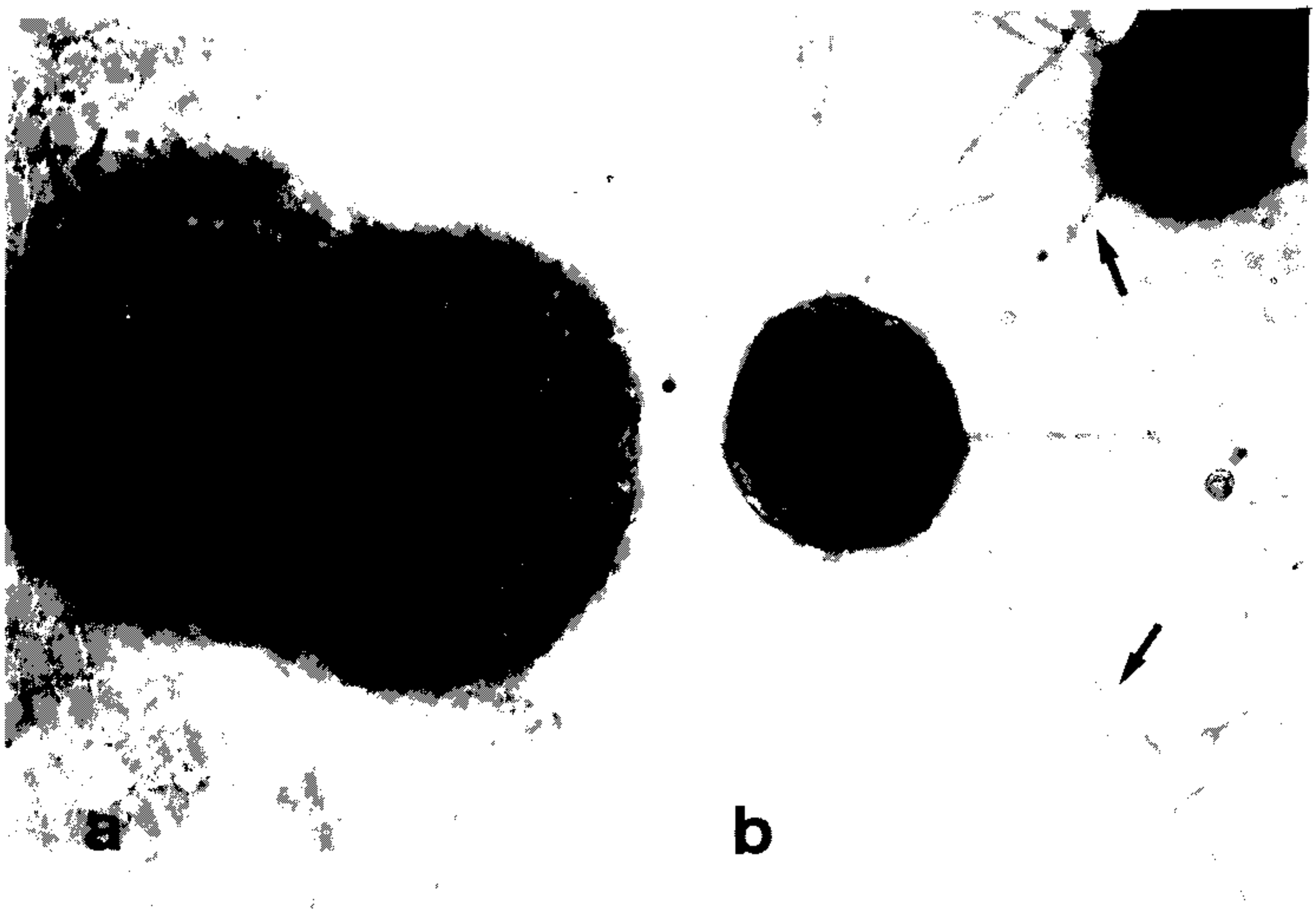

Fig. 1: (a) Negatively stained *N. lactamica* 244 from primary culture of a carrier. Cells were devoided of pili. Note bleblike evaginations of outer membrane (arrow). 90000X. (b) Piliated *N. meningitis* included for comparison. Several pili around cells are seen (arrows). 85000X.

RESUMO

As secreções nasais de voluntários colonizados por *N. lactamica* inibem a aderência de *N. lactamica* e de meningococos dos grupos A e B à células oroepiteliais humanas, "in vitro". A aderência, nas amostras de *Neisseria* testadas, decorre da presença de adesinas não-fimbriais que parecem possuir antígenos comuns. A despeito do surgimento de marcante atividade antiadesiva nas secreções nasais os voluntários persistiram como portadores de *N. lactamica.* A erradicação do estado de portador parece assim ser dependente da ação de diferentes fatores.

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REFERENCES

ANDRADE, J.R.C.; WANDERSMAN, A. & SANTA ROSA, M.R., 1983. Pili and adhesive properties in *Neisseria meningitidis.* Resumos do 9º Congresso Latino-Americano de Microbiologia, p. 86. São Paulo.

BOIA, M.N.; ANDRADE, J.R.C. & CAMILLO-COURA, L., manuscript in preparation. Prevalência de *Neisseria meningitidis* e *Neisseria lactamica,* em crianças faveladas, na cidade do Rio de Janeiro.

CAUDURO, P.F.; MEZZARI, A. & DIAS, C.A.G., 1984. Conjuntivite por *Neisseria lactamica. Rev. Microbiol.* (S. Paulo), *15* :227-230.

CORBETT, W.P. & CATLIN, B.W., 1968. Galactosidase activity of lactose-positive *Neisseria. J. Bacteriol., 95* :52-57.

FUBARA, E.S. & FRETER, R., 1973. Protection against enteric bacterial infection by secretory IgA antibodies. *J. Immunol., 111* :395-403.

GOLD, R.; GOLDSCHNEIDER, I.; LEPOW, M.L.; DRAPER, T.F. & RANDOLPH, M., 1978. Carriage of *Neisseria meningitidis* and *Neisseria lactamica* in infants and children. *J. Infect. Dis., 137* :112-121.

HÖEFFLER, D.F., 1974. Recovery of *Neisseria meningitidis* from the nasopharynx. Comparison of two techniques. *Am. J. Dis. Child., 128* :54-56.

HOLLIS, D.G.; WIGGINS, G.L.; WEAVER, R.E. & SCHUBERT, J.M., 1970. Current status of lactose-fermenting *Neisseria*. *Ann. N.Y. Acad. Sci., 174* :444-449.

LAUER, B.A. & FISHER, C.E., 1976. *Neisseria lactamica* meningitis. *Am. J. Dis. Child., 130* :198-199.

LEE, I.M.L.; PESSÔA, G.V.A., ESPER, M.R.N.R.; MELLES, C.E.A. & SIMONSEN, V., 1978. Importância da pesquisa da beta-galactosidase na caracterização laboratorial de *Neisseria lactamica*. *Rev. Inst. Adolfo Lutz, 38* :23-27.

MORELLO, J.A.; JANDA, W.M. & BOHNHOFF, M., 1985. *Neisseria* and *Branhamella*. *In:* Manual of Clinical Microbiology. Lennette, E.N. et al. American Society for Microbiology, Washington, D.C. 4th edition, 176-192.

STEPHENS, D.S.; HOFFMAN, L.H. & McGEE, Z.A., 1983. Interaction of *Neisseria meningitidis* with human nasopharyngeal mucosa: attacment and entry into columnar epithelial cells. *J. Infect. Dis., 148* :369-376.

STEPHENS, D.S. & McGEE, Z.A., 1981. Attachment of *Neisseria meningitidis* to human mucosal surfaces: influence of pili and type of receptor cell. *J. Infect. Dis., 143* :525-532.

STEPHENS, D.S.; McGEE, Z.A.; MELLY, M.A.; HOFFMAN, L.H. & CREGG, C.R., 1982. Attachment of pathogenic *Neisseria* to human mucosal surfaces: role in pathogenesis. *Infect. Immun., 10* :192-195.

SUASSUNA, I.; LOURENÇO, N.J.; ANDRADE, J.R.C. & PEREIRA, A.A., 1975. *Neisseria lactamicus* em portadores. Causa de erro no diagnóstico de portador de meningococos. *Rev. Bras. Patol. Clin., 11* :32.

THAYER, J.D. & MARTIN, J.E., 1964. A selective medium for the cultivation of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. *Publ. Hlth. Rep., 79* :49-57.

WENZEL, R.P., MITZEL, J.R.; DAVIES, J.A. & BEAM JR., W.E., 1972. Serum and nasal secretion immune response in meningococcal disease. *Infect. Immun., 5* :627-629.

WILSON, H.D. & OVERMAN, T.L., 1976. Septicemia due to *Neisseria lactamica*. *J. Clin. Microbiol., 4* :214-215.